1.04-5.58, p = 0.04) when compared with UCB, but not with BM as cell source (p = 0.17).

This retrospective single institutional study of 628 consecutive allogeneic transplantation patients revealed some novel findings. PSC were associated with a higher incidence of aGVHD, but not cGVHD. The combination of FK/MTX was associated with a lower risk of cGVHD than CSA/MTX and will need to be investigated further. Validation of these findings requires large cooperative prospective studies.

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TNFα -238A ALLELE IDENTIFIES PATIENTS WHO DEVELOP BOTH ACUTE AND CHRONIC GVHD AFTER MATCHED UNRELATED DONOR TRANSPLANT IN CHILDREN: A PEDIATRIC BLOOD AND MARROW TRANSPLANT CONSORTIUM STUDY

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Introduction: The inflammatory cytokine tumor necrosis factor-α (TNFα) plays a central role in the pathogenesis of acute GVHD, but its role in chronic GVHD is less clearly defined. We recently described an association between the recipient TNFα gene polymorphisms and the severity of acute GVHD in pediatric unrelated donor BMT (Goyal et al, *Biol Blood Marrow Transplant*, 2010). We now report the correlative analyses between the recipient and donor TNFα promoter region single nucleotide gene polymorphisms (SNP) and the risk of acute and chronic GVHD in this cohort.

Materials and Methods: Genotyping was performed on pretransplant genomic DNA samples from recipient-donor pairs (n = 180). To address the confounding effect of population stratification, significant associations were reanalyzed in white recipient-donor pairs.

Results: Twenty-three patients died before day+100; 78/153 (51%) of the remaining evaluable patients developed cGVHD with extensive disease in 60/76 (79%) patients. Similar to findings in recipients, the donor TNFα variant A allele of -863C/A SNP (HR 3.67, p = 0.01) and the variant C allele of -1031T/C SNP (HR 2.85, p = 0.05) were also associated with grade III-IV aGvHD. The recipient TNFα variant A allele of - 238G/A SNP was associated with grade II-IV aGVHD (HR 2.38; p<0.01, previously reported) as well with cGVHD (RR 1.68; P = 0.02). Six out of 44 (14%) patients (14%) with variant -238A allele compare to 40/99 (40%) patients without -238A allele did not develop acute or chronic GVHD. The rates of only acute (16%, 18%) and only chronic GVHD (18%, 19%) were similar in those with or without recipient -238A allele, respectively. However, 23/44 (52%) patients with the -238A allele developed both acute and chronic GVHD compared with 22/99 (22%) without the -238A allele (RR 2.35, p<0.01, Table 1). These associations remained significant when analyzed in white-only recipient-donor pairs. No statistically significant association was detected between the donor TNFα gene polymorphisms and the risk of cGVHD.

Table 1.

Recipient TNF alpha -238 G>A	No Acute or Chronic GVHD	Only Acute GVHD	Only Chronic GVHD	Both Acute & Chronic GVHD	P-Value
AA/AG	6/14 (14%)	7/44 (16%)	8/44 (18%)	23/44 (52%)	<0.01
GG	40/99 (40%)	18/99 (19%)	19/99 (19%)	22/99 (22%)	

Conclusions: In this large cohort of pediatric matched unrelated donor transplants: 1) The recipient and donor TNFα -863A allele and -1031C allele are associated with grade III-IV aGvHD. 2) The recipient TNFα -238A allele identifies a subset of patients who develop both acute and chronic GVHD. These findings deserve further study in independent cohorts and may be clinically relevant in a risk-adjusted approach to GVHD management in pediatric unrelated donor transplants.

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COMPARISON OF SIROLIMUS AND MYCOPHENOLATE MOFETIL AS SALVAGE TREATMENT FOR ACUTE GRAFT-VERSUS-HOST DISEASE

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Glucocorticoid refractory acute GVHD (aGVHD) is a major source of mortality following allogeneic HCT. Comparative studies to evaluate the efficacy of salvage immune suppressive agents are lacking. We retrospectively compared the efficacy of sirolimus (SIR) and mycophenolate mofetil (MMF) as salvage aGVHD therapy for glucocorticoid refractory, dependent or intolerant patients. Of 281 consecutive patients who received allogeneic HCT from 07/2004 to 09/2009, we identified 84 patients who received tacrolimus/methotrexate (Tac/MTX) GVHD prophylaxis, were treated with glucocorticoids for grades 2-4 aGVHD, were refractory (n = 72) or dependent (n = 12) to glucocorticoids, and received 2nd line GVHD treatment with MMF (n = 56) or SIR (n = 28). Demographics and treatment variables were similar except for year of transplant (earlier for MMF). Disease diagnoses included AML (n = 27), NHL (n = 14), MDS (n = 12), ALL (n = 9), CML (n = 7), CLL (n = 4), SAA (n = 2), MPD (n = 5), MM/PCL (n = 3), and HL (n = 1). Conditioning regimens were busulfan/fludarabine for 71, and other regimens for 13. Except for 1 bone marrow graft in each group, all received peripheral blood stem cells. Graft sources were from HLA-matched siblings (35), or 8/8 HLA-matched unrelated donors (49). Overall grade distribution of aGVHD at time of salvage for MMF vs. SIR was the following: grade 1 (13 vs. 2), grade 2 (31 vs. 16), grade 3 (9 vs. 5) and grade 4 (3 vs. 5). Median steroid dose at the time of salvage was 1 (range 0.17 – 2.28) mg/kg for MMF group and 1 (range 0.12 – 2.0) mg/kg for SIR group. Median time from steroid to salvage was 20 (range 1 – 208) days for MMF and 19 (range 1 – 275) days for SIR (p = 0.84). Complete response (CR) rates following initiation of MMF or SIR did not significantly differ at the following time points: 1 week (MMF 30%, SIR 21%), 4 weeks (MMF 44%, SIR 46%), and 6 weeks (MMF 60%, SIR 58%). Overall response rates (ORR) also did not differ significantly: 1 week (MMF 57%, SIR 42%), 4 weeks (MMF 57%, SIR 77%), and 6 weeks (MMF 72%, SIR 75%). Flare or progression of aGVHD while on salvage regimen was noted in 50% (MMF) and 36% (SIR) of patients (p = 0.64). Median overall survival from the time of salvage therapy for MMF vs. SIR did not significantly differ, 11.6 (95% CI 7.0 – 28.1) vs. 9.7 (95% CI 5.4 – 15.9) months, log-rank p = 0.88. These retrospective data suggest that MMF and SIR have comparable activity in the treatment of steroid refractory or dependent acute GVHD.

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HUMAN MULTIPOTENT ADULT PROGENITOR CELLS EFFECTIVELY MODULATE ALLOREACTIVITY AFTER BONE MARROW TRANSPLANTATION REDUCING GVHD WHILE PRESERVING GRAFT-VERSUS-LEUKEMIA ACTIVITY

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Graft-versus-host disease (GVHD) limits successful outcomes following allogeneic BMT (allo-BMT). The pathophysiology of GVHD involves three distinct phases which contribute to inflammatory cytokine dysregulation, the generation of cellular effectors, and target organ injury. This framework uncovers opportunities to regulate GVHD. We examined whether reported immunosuppressive and regenerative properties of human, bone marrow-derived multi-potent, adult progenitor cells (hMAPCs) could regulate GVHD using established murine models. The immuno-regulatory

capacity of MultiStem® (Athersys, Inc), a commercial hMAPC product, was first evaluated *in vitro* in a MLC of purified mouse dendritic and T cells. MultiStem® inhibited mouse T-cell proliferation in a reproducible, dose dependent fashion not observed with human dermal fibroblasts. This effect was associated with reduced T cell activation and inflammatory cytokine secretion and robust increases in the concentrations of PGE2 and TGFb in cell culture supernatants. Importantly, MultiStem® had no effect on the generation CTL activity *in vitro*. Similar findings were observed *in vivo* when MultiStem® was delivered in the context of allo-BMT using C57BL/6 and B6D2F1 mice as donors and recipients, respectively. Tail vein injection of MultiStem® on Day +1 (D1) and +4 (D4) resulted in significant reductions in splenic T cell expansion and in numbers of TNFa and IFNg-producing CD4+ and CD8+ cells at D10 compared to untreated allo controls. These findings were associated with reductions in 1) D10 serum levels of TNFa and IFNg, 2) D10 intestinal and hepatic inflammation and 3) systemic GVHD as measured by survival and clinical score. Suppression of systemic GVHD was observed in a dose dependent manner. Biodistribution studies using bioluminescence and cryo-imaging showed that MultiStem® tracked from the lung and into the abdominal organs (liver and spleen) within the first 4 days after injection. When GVL effects were tested, the administration of MultiStem® resulted in superior leukemia free survival; MultiStem® treated mice had less GVHD but effectively eradicated tumor challenge. Collectively, these data provide critical information regarding the biodistribution and regulatory capacity of human MultiStem® in established models of GVHD. Mechanistic studies are ongoing, which we anticipate will be useful in optimizing the dose and schedule of MultiStem® in ongoing, first-in-human clinical trials following allogeneic BMT.

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SEVERE DENTAL CARIES IN PATIENTS WITH ORAL CHRONIC GRAFT-VERSUS-HOST DISEASE

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Objective: The oral cavity is one of the most frequently affected sites by cGVHD following allogeneic hematopoietic cell transplantation (alloHCT), and can be a significant source of patient morbidity due to both mucosal and salivary gland involvement. The development of dental decay is a potentially devastating oral complication that has only rarely been reported in the transplantation literature. The purpose of this study was to comprehensively characterize a cohort of patients with cGVHD that subsequently developed extensive dental caries.

Methods: A retrospective case-record review was conducted for patients who had undergone alloHCT at Dana-Farber/Brigham and Women's Cancer Center between 1990 and 2010 and developed cGVHD-associated rampant dental decay. All patients underwent dental evaluations before and after alloHCT that consisted of a soft and hard tissue examination and dental radiographs. Caries diagnosed from the pre-alloHCT evaluation were treated definitively such that all patients were considered free of caries at the time of admission for HCT.

Results: A total of 21 patients were identified with a median time of cGVHD onset of 5.4 months (range 2.2-18.5) post-alloHCT. All patients were diagnosed with oral cGVHD, with 90% demonstrating mucosal involvement, and 95% with salivary gland involvement. Post-alloHCT dental evaluation was performed at a median of 22 months (range 4-81) after alloHCT. when ten patients were diagnosed with gross caries and eight patients presented four or more affected teeth. Cervical and interproximal patterns of dental caries were frequently diagnosed. The proportion of patients with gross, one surface, and greater than one surface caries, categorized as 0, 1-3, or ≥4, were significantly higher post-alloHCT compared to pre-alloHCT with at least 50% of patients experiencing an increase.

Conclusions: Patients with oral cGVHD, who were free of caries at the time of transplantation, developed extensive areas of cervical decay in a median time of less than two years post-alloHCT. This is the first comprehensive characterization of this severe late complication of alloHCT and oral cGVHD. Greater awareness on the part of transplant oncologists and dentists as well as more aggressive preventive measures are warranted, as well as prospective studies to better elucidate the incidence of this complication, identify risk factors, and evaluate the effectiveness of preventive interventions.

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IN VIVO REFLECTANCE CONFOCAL MICROSCOPY IN THE EARLY DIAGNOSIS OF ACUTE CUTANEOUS GRAFT-VERSUS-HOST DISEASE: A PILOT STUDY

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Background: Graft-versus-host disease (GVHD) is a common and potentially life-threatening complication of allogeneic hematopoietic stem cell transplantation. The diagnosis of cutaneous GVHD can be challenging, as it is based on clinical presentation, which often simulates other processes. Early recognition and treatment are essential to reduce the associated morbidity and mortality. Reflectance confocal microscopy (RCM) is a high-resolution, noninvasive technique that may facilitate a timely diagnosis and prompt institution of treatment.

***Objective*:** We sought to describe the features of acute cutaneous GVHD under RCM and to correlate these with the histopathological findings.

***Methods*:** Patients were recruited prospectively to undergo clinical and RCM examination. A total of 19 patients were enrolled, five of whom developed acute cutaneous GVHD following an allogeneic hematopoietic stem cell transplant. RCM images from these five cases were examined and the key confocal features were compared to histopathological findings.

Results: On RCM examination, features of acute cutaneous GVHD, such as apoptotic keratinocytes, inflammatory cell infiltration and interface dermatitis, were identified and were shown to correlate well with histopathological findings.

***Limitations*:** The open-label, unblinded nature of this study allows for potential observer bias and the small sample size may not reflect the findings in a larger population. The sensitivity and specificity of RCM in this population remain to be determined.

***Conclusion*:** The findings support further examination of the use of RCM in the rapid, bedside diagnosis of acute cutaneous GVHD.

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SIGNIFICANT ADVERSE EFFECTS ASSOCIATED WITH SIROLIMUS/TACROLIMUS GVHD PROPHYLAXIS REGIMEN (SIRO-tac) IN ALLOGENEIC HEMATOPOEITIC STEM CELL TRANSPLANT (alloHSCT) PATIENTS (pts): A SINGLE INSTITUTION EXPERIENCE

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Background: On account of the increased transplant related morbidity and mortality with the use of methotrexate (MTX) based immunosuppressive therapy we adopted a new policy in GVHD prophylaxis using a combination of tacrolimus and sirolimus aborting the use of MTX. We report the adverse effects that were noted in our patients who underwent a related or un-related AlloHSCT transplantation.

Patients and Methods: Data from 46 consecutive AlloHSCT pts over a 2 year period were reviewed. 39 pts who received siro-tac with or without ATG were identified. Demographics, indication for transplantation, type of donor, conditioning regimen (CR), GVHD prophylaxis associated complications were identified and collected.

Results: 46 pts underwent an alloHSCT at our institution from 2009-11. 39 (84.78%) pts (average age 44 yrs, range 20-65 yrs) received Siro-tac with or without ATG and all without MTX. 21 (53.84%) pts were male and 18 (46.15%) pts female. Indications for transplant included relapsed non-Hodgkin lymphoma, Hodgkin lymphoma, acute myelogenous leukemia, acute and chronic lymphocytic leukemia, myelodysplastic syndrome and myelofibrosis. 15 (46.15%) pts received a myeloablative CR. 14 pts had an unrelated